Institute of Paper Science and Technology
Atlanta, Georgia

IPST Technical Paper Series Number 787

Pulping, Bleaching, and Characterization of CAD-Deficient Wood

D.R. Dimmel, J. MacKay, E. Althen, and C. Parks

May 1999

Submitted to
The Tenth International Symposium on Wood and Pulping Chemistry
Yokohama, Japan
June 7-10, 1999

INSTITUTE OF PAPER SCIENCE AND TECHNOLOGY
PURPOSE AND MISSIONS

The Institute of Paper Science and Technology is an independent graduate school, research organization, and information center for science and technology mainly concerned with manufacture and uses of pulp, paper, paperboard, and other forest products and byproducts. Established in 1929, the Institute provides research and information services to the wood, fiber, and allied industries in a unique partnership between education and business. The Institute is supported by 52 North American companies. The purpose of the Institute is fulfilled through four missions, which are:

- to provide a multidisciplinary education to students who advance the science and technology of the industry and who rise into leadership positions within the industry;
- to conduct and foster research that creates knowledge to satisfy the technological needs of the industry;
- to serve as a key global resource for the acquisition, assessment, and dissemination of industry information, providing critically important information to decision-makers at all levels of the industry; and
- to aggressively seek out technological opportunities and facilitate the transfer and implementation of those technologies in collaboration with industry partners.

ACCREDITATION

The Institute of Paper Science and Technology is accredited by the Commission on Colleges of the Southern Association of Colleges and Schools to award the Master of Science and Doctor of Philosophy degrees.

NOTICE AND DISCLAIMER

The Institute of Paper Science and Technology (IPST) has provided a high standard of professional service and has put forth its best efforts within the time and funds available for this project. The information and conclusions are advisory and are intended only for internal use by any company who may receive this report. Each company must decide for itself the best approach to solving any problems it may have and how, or whether, this reported information should be considered in its approach.

IPST does not recommend particular products, procedures, materials, or service. These are included only in the interest of completeness within a laboratory context and budgetary constraint. Actual products, procedures, materials, and services used may differ and are peculiar to the operations of each company.

In no event shall IPST or its employees and agents have any obligation or liability for damages including, but not limited to, consequential damages arising out of or in connection with any company's use of or inability to use the reported information. IPST provides no warranty or guaranty of results.

Pulping, Bleaching, and Characterization of CAD-Deficient Wood

D. R. Dimmel, J. MacKay, E. Althen, C. Parks, Institute of Paper Science and Technology, 500 10th Street NW, Atlanta, Georgia 30318 (U.S.A.)
E-mail: donald.dimmel@ipst.edu

J. J. Boon, FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands

ABSTRACT

One approach to improve the selectivity of pulping is to use wood that is rich in strong fibers and is easily delignified. To this end, considerable interest has developed in a mutant loblolly pine tree that is deficient in cinnamyl alcohol dehydrogenase (CAD). The absence of the CAD enzyme leads to a different pool of precursors for lignin production. Structural studies, including characterization by pyrolysis GC-MS and UV presented here, indicate increased levels of coniferaldehyde, dihydroconiferyl alcohol, and vanillin units, and lower levels of coniferyl alcohol in the CAD-deficient lignin as compared to normal pine lignin. Wood from a 12-year-old CAD-deficient loblolly pine has been pulped under soda, kraft, and soda/AQ conditions. In comparison to a normal 12-year-old loblolly pine, the CAD-deficient wood was much more easily delignified. The high reactivity of CAD-deficient wood may be related to the presence of less cross-linked, lower molecular weight lignin. The molecular weight of an isolated milled wood lignin from CAD- was ~50% less than that from a normal pine tree. The molecular weights of lignins isolated from soda pulping liquors for CAD-deficient and normal pine cooks were quite similar.

INTRODUCTION

Genetic manipulation of lignin biosynthesis in trees is a target in several research groups aiming to facilitate lignin removal during the pulping and bleaching. Trees containing easily extracted lignin will pulp more rapidly or under milder conditions, leading to increased productivity, lower energy, lower bleaching costs and by-products, as well as increased yield due to reduced carbohydrate degradation. Manipulation of lignin has been achieved in poplar by genetic transformation (introduction of foreign genes);[1] potential benefits for pulping have been shown in small-scale cooks. However, genetic transformation of commercial softwoods is not yet possible on a routine basis.

To better understand the structure of genetically modified lignins, we must first consider the production of normal lignin monomers. The biosynthesis of lignin involves converting phenylalanine to three primary cinnamic acid intermediates **1-3** (Figure 1).[2,3] Softwood lignins are principally derived from ferulic acid (**2**), while hardwood lignins are derived from both **2** and **3**.[4,5] The primary precursors are selectively reduced to aldehydes (**4**) and then further to alcohols, coniferyl alcohol (**5**), in the case of softwoods (Figure 1). The latter step requires the cinnamyl alcohol dehydrogenase (CAD) enzyme.[6,7]

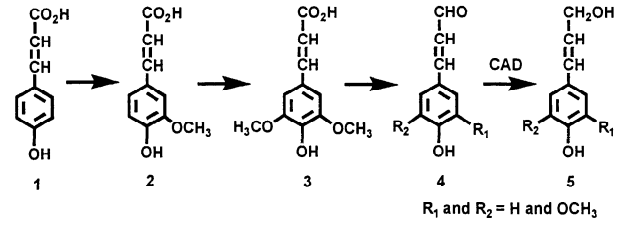

Figure 1. Biosynthesis pathway for production of normal lignin monomer building blocks.

Lignification begins with the oxidation of coniferyl alcohol to a radical; several resonance forms of the radical exist. The radicals couple with one another to build up a polymer network. The preferred coupling involves union of an O_4-radical with a C_β-radical; approximately 50% of the interunit linkages in softwood lignin are of this type (Figure 2).[8] Several other linkages are also present in varying amounts, including C_5-C_5 (Figure 2), C_1-C_β, C_β-C_β, C_5-O_4, etc.[8]

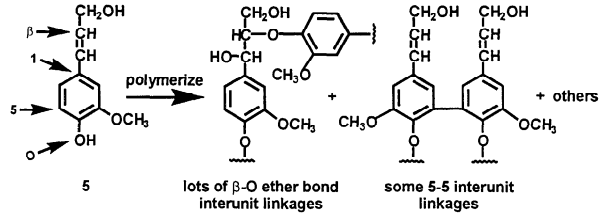

Figure 2. Polymerization of normal lignin monomer building blocks.

Loblolly pines with unusual lignin can be produced without using genetic transformation.[9,10] CAD-deficient pines have been obtained through crosses of trees that have a mutant gene, the *cad-n1* allele, found in breeding stocks of loblolly pine. CAD-deficient trees have lignin properties of potential commercial value. The *cad-n1* allele can be used in well-defined crosses to produce: (a) trees almost *completely deficient* in CAD activity, and (b) trees *partially deficient* in CAD activity (~50% of normal).

Lignins from completely CAD-deficient trees are built up from unusual monomers. Analysis of milled wood lignins by NMR techniques indicate that coniferaldehyde (**4**), vanillin (**6**), and dihydroconiferyl alcohol (**7**) are the principal monomers (Figure 3).[10] The changes to the monomer pool are more dramatic than in genetically engineered plants or trees with decreased CAD activity, described so far.[1,11-13] Relative to normal pinewood, lignin in these CAD-deficient trees contains much less C_β-O_4 linkages and high amounts of C_5-linkages.[14] Such linkage distribution is consistent with lignin biosynthesis theory and the type of monomers available in the CAD- case.

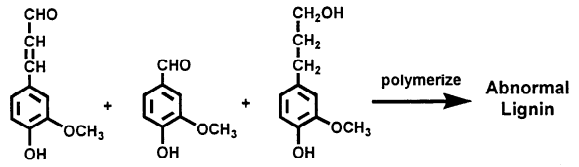

Figure 3. The unusual monomer building blocks for lignin production in CAD-deficient trees.

Linkages involving C_β are not possible with CAD-deficient lignin precursors **6** and **7**, and probably low in frequency with precursor **4**. One can speculate that C_1-linkages will also be much less since the aldehyde group at C_1 in the precursor **6** and the saturated side-chain at C_1 in precursor **7** will not be easily lost following radical coupling to these sites.

Coniferyl alcohol (**5**), the building block of normal lignin, has four available radical reaction sites (O_4, C_5, C_1, and C_β). Except for coniferaldehyde (**4**), the unusual CAD-deficient precursors only have two reactive radical sites (O_4 and C_5). Because of the low number of reactive sites and the lack of active C_β-sites in the precursors, the lignin in totally CAD-deficient pines will likely be less cross-linked, be inhibited in polymer growth, have a lower molecular weight and, thus, be more easily dissolved in alkali. Our studies are directed at establishing this point.

RESULTS AND DISCUSSION

Pulping of CAD-Deficient Wood

Our previous studies have shown that large amounts of lignin are removed from totally CAD-deficient wood simply by mild alkaline treatment at room temperature.[15] We have recently conducted an extensive study of the soda, soda/AQ, and standard kraft pulping of normal and totally CAD-deficient wood. Wood samples were taken from 12-year-old trees, grown on the same site.

One of the first challenges that we had to address was conducting a large number of cooks with just 400 grams of dry CAD-deficient chips. The initial months of our study concerned showing that small scale (0.5 g) cooks and mini-kappa number determinations gave results similar to 1 kg cooks and regular kappas. The pulping studies were performed using 4-mL pressure vessels in a fluidized sand bath with automated temperature control.

Our research has shown that CAD- pines are easily pulped under soda and kraft conditions. Data for the soda pulping of CAD- and normal wood with 18% NaOH at several different H-factors (cook severities) is shown in Figure 4. The amount of delignification depended much more on the H-factor and than the NaOH level of the cook. The relatively low response of the normal wood to changes in the H-factor is probably related to the use of a 7:1 liquor-to-wood ratio while maintaining the absolute amounts of NaOH and NaSH the same as a 4:1 cook. Performing cooks this way meant that NaOH and NaSH concentrations were less than normally employed in a more typical 4:1 cook. The 7:1 liquor-to-wood ratio was needed because the swelled chips in the small bombs were basically void of bulk liquor if a standard 4:1 liquor-to-wood ratio was used.

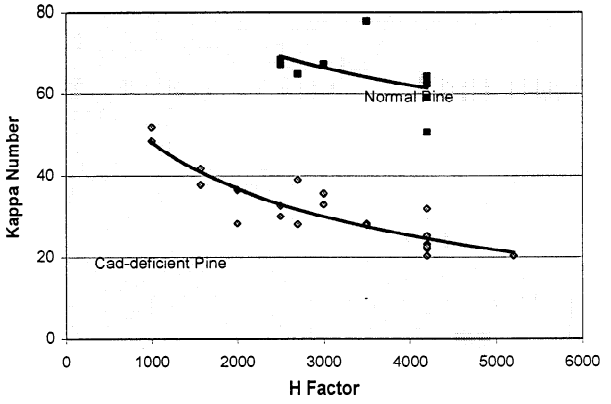

Figure 4. Relationship between kappa number and H factor (energy input) for pulps obtained from 18% NaOH cooks of CAD-deficient and normal loblolly pine chips, using a 7:1 liquor-to-wood ratio; absolute amounts of NaOH and NaSH were the same as a 4:1 cook.

Similar findings (as shown in Figure 4) were observed with kraft pulping when the chemical concentrations of NaOH and NaSH were moderate. However, if the concentration of NaOH and NaSH were high, we observed little difference in the degree of delignification of CAD- and normal wood (Figure 5). Delignification occurs in three stages: initial, bulk, and residual. We interpret our results to mean that, in the CAD-deficient case, the initial and bulk phase reaction rates are quite fast, but the residual phase rate is slow, similar to normal wood. For harsh cooks, each wood delignifies down to roughly the same level of residual lignin.

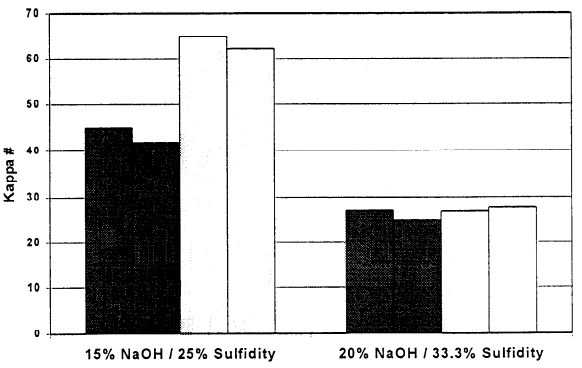

Figure 5. Relationship between kappa number and cook chemical composition for duplicate CAD-deficient (dark-colored bars) and normal (light-colored bars) loblolly pine pulping, using a 7:1 liquor to wood ratio and a 2000 H-factor in each case.

The comparison of kappa numbers from CAD- and control pine could be a problem if the CAD- and control lignins had different reactivities towards $KMnO_4$. Since CAD- lignin presumably contains high amounts of aldehyde, we might expect less consumption of $KMnO_4$. This concern was dispelled when Jiebing Li graciously performed $KMnO_4$ consumption tests[16] of CAD- and control isolated milled wood lignins and showed no significant differences.

Isolating the lignins from the pulping liquors provided another piece of supporting evidence for greater lignin loss in the CAD- case. The CAD- liquors contained higher amounts of dissolved lignins. At the same H-factor, there was ~50% more dissolved lignin in the CAD- case. Lignins isolated from CAD- and control pine cooking liquors displayed nearly the same molecular weights, regardless of the length of the cooks. It should be noted, however, that the isolated acetylated lignins displayed variable solubility in organic solvents, rendering doubts on the molecular weight determinations.

There is a much lower pulp yield in the CAD- case, which we attribute to its juvenile state. A completely CAD- tree does not grow as well as a normal tree. A twelve-year-old tree looks more like a seven-year-old tree. Our interest in completely CAD-deficient trees is more academic than practical. Understanding the reactivity of CAD- lignin could lay the groundwork for defining what type of lignin would be good to engineer into new trees.

Lignin Characterization

Several studies have characterized CAD- lignin. Thioacidolysis[14] and NMR[12] studies indicate that the CAD- lignin has much fewer β-aryl ether linkages and more 5-5 linkages than normal pine. Both techniques also indicate increased levels of coniferaldehyde and dihydroconiferyl alcohol lignin subunits. Comparative pyrolysis gas chromatography mass spectrometry (pyGC/MS) studies of CAD- and normal pinewood support these conclusions. The normal wood has a dominant signal at *m/e* 180 in its electron impact (EI) pyGC/MS that corresponds to coniferyl alcohol. The CAD- wood shows *m/e* 178, 180, and 182 signals of comparable intensities. The former signal corresponds to coniferaldehyde and the latter to dihydroconiferyl alcohol. Further confirmation of these assignments comes from ammonia chemical ionization techniques.

Two UV studies have been performed that indicate a major change in the type and/or abundance of phenolic components in the CAD- wood. We have recorded solution UV spectra of normal and CAD- milled wood lignins (MWL) from two 12-year-old trees. The UV spectra of the two MWLs were very distinct from one another (Figure 6). They both had an absorption maximum at 280, typical for pine MWL; however, the *cad-n1* MWL had a broad shoulder extending to 350 nm. Sodium borohydride reduction, which selectively reduces carbonyl groups, eliminated this shoulder, indicating that it was likely due to conjugated carbonyl groups. This interpretation of the UV spectra and the effect of borohydride reduction are consistent with the increased proportion of coniferaldehyde and vanillin subunits detected in the CAD-deficient wood by FTIR[9] and NMR spectroscopy of MWLs.[10]

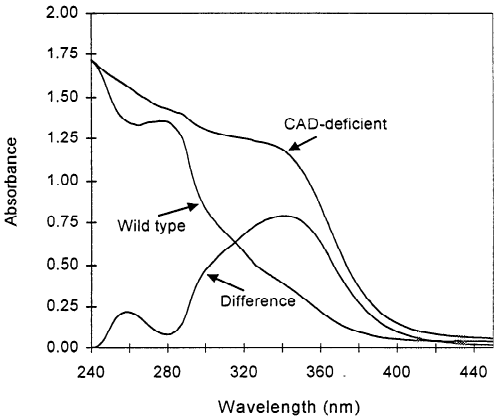

Figure 6. UV spectra of MWL from wild type and CAD-deficient pine trees in 90% dioxane:water.

In addition, we have used UV microspectrophotometry to characterize the lignin in sections of wood.[17] This technique directly measures the UV absorbance spectrum of cell bound phenolics and avoids the issue of structural alterations due to lignin isolation. The control normal pine displayed an expected[18] absorbance maximum at 280 nm and a relatively low absorbance at wavelengths above 300 nm. In contrast, the CAD- wood had a maximum at 280 nm, but a large increase in absorbance between 300-350 nm. The altered spectra of the CAD- woods were consistent with the CAD- MWL spectrum.

Finally, milled wood lignin obtained from CAD- wood is ~50% lower in molecular weight than that obtained from normal pine; this 50% difference was also confirmed by an outside laboratory (Figure 7).

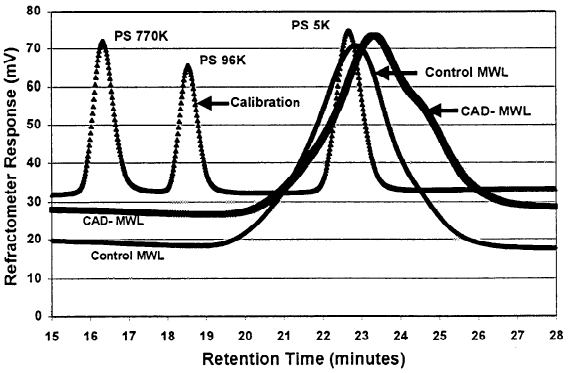

Figure 7. Comparison of the molecular distributions of CAD-deficient and normal milled wood lignins with polystyrene standards.

CONCLUSIONS

With a higher amount of 5-5 lignin linkages, CAD-wood should be more difficult to delignify than normal wood. But just the opposite is found. Totally CAD-deficient loblolly wood is much more easily delignified than normal pine. The lignin in the CAD-pine is lower in molecular weight. This fact may be a principal reason for the easy lignin removal; it takes fewer cleavages to get a water-soluble piece. Another factor could be that the lignin is less cross-linked. The nature of the monomers making up the lignin suggests there should be low amounts of cross-linking, but higher amounts of C_5-linkages. Since linkages to C_5 are difficult to break, the last stages of lignin removal (bleaching) may be difficult.

We are presently attempting to compare the bleachability of pulps of similar kappa numbers derived from CAD-deficient and normal wood. Another direction of our research is to explore the pulping and bleaching of partially CAD-deficient loblolly pines and the corresponding lignin structure. Partially deficient trees show an increase of 14% in debarked volume after 4 years of growth in comparison to the normal tree.[19]

REFERENCES

1. Baucher, M., Chabbert, B., Pilate, G., van Doorsselaere, J., Tollier, M.-T., Petit-Conil, M., Cornu, D., Monties, B., van Montagu, M., Inzé, D., Jouanin, L., and Boerjan, W. Red xylem and higher lignin extractability by down-regulating cinnamyl alcohol dehydrogenase in poplar (*Populus tremula x Populus alba*). *Plant Physiol.*, 112, 1479-1490 (1996).
2. Whetten, R. W., and Sederoff, R. R. Lignin biosynthesis. *Plant Cell*, 7, 1001-1013 (1995); Whetten, R. W., MacKay, J. J., and Sederoff, R. R. Recent advances in lignin biosynthesis. *Ann. Rev. Plant Physiol. & Plant Mol. Biol.*, 49, 585-609 (1998).
3. Boudet, A.M., Lapierre, C., and Grima-Pettenati, J. Biochemistry and molecular biology of lignification. *Tansley Review No. 80, New Phytol.*, 129, 203-236 (1995).
4. Monties, B. Lignins. in Methods in Plant Biochemistry, Editors, Dey, P. M., and Harborne, J. B., Academic Press, London, Vol. 1, pp. 113-158 (1989).
5. Adler, E. Lignin chemistry - past, present and future. *Wood Sci. Technol.*, 11; 169-218 (1977).
6. MacKay, J. J., Liu, W., Whetten, R. W., Sederoff, R. R., and O'Malley, D. M. Genetic analysis of cinnamyl alcohol dehydrogenase (*CAD*) in loblolly pine: single gene inheritance, molecular characterization and evolution. *Molecular and General Genetics*, 247, 537-545 (1995).
7. O'Malley, D., Porter, S., and Sederoff, R. R. Purification, characterization and cloning of cinnamyl alcohol dehydrogenase in loblolly pine (*Pinus taeda* L.). *Plant Physiol.*, 98, 1364-1371 (1992).
8. Sjostrom, E. Wood Chemistry Fundamentals and Applications, 2nd Edition, Academic Press, San Diego, CA, 1993, Chapter 4.
9. MacKay, J. J., O'Malley, D. M., Presnell, T., Booker, F. L., Campbell, M. M., Whetten, R. W., and Sederoff, R. R. Inheritance, gene expression and lignin characterization in a mutant pine deficient in cinnamyl alcohol dehydrogenase. *Proc. Natl. Acad. Sci. USA*, 94, 8255-8260 (1997).
10. Ralph, J., MacKay, J. J., Hatfield, R. D., O'Malley, D. M., Whetten, R. W. and Sederoff, R. R. An abnormal lignin in a mutant loblolly pine (*Pinus taeda* L.). *Science*, 277, 235-239 (1997).
11. Halpin, C., Knight, M. E., Foxon, G. A., Campbell, M. M., Boudet, A. M., Boon, J. J., Chabbert, B., Tollier, M.-T., and Schuch, W. Manipulation of lignin quality by downregulation of cinnamyl alcohol dehydrogenase. *Plant Journal*, 6, 339-350 (1994).
12. Ralph, J., Hatfield, R. D., Piquemal, J., Yahiaoui, N., Pean, M., Lapierre, C., Grima-Pettenati, J., and Boudet, A. M. NMR characterization of altered lignins extracted from tobacco plants down-regulated for lignification enzymes cinnamyl-alcohol dehydrogenase and cinnamoyl-CoA reductase. *Proc. Nat. Acad. Sci.*, 95, 12803-12808 (1998).
13. Lapierre, C., Pollet, B., Petit-Conil, M., Toval, G., Romero, J., Pilate, G., Leple, J.-C., Boerjan, W., Ferret, V., De Nadai, V., and Jouanin, L. Structural alteration of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid O-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiol.*, 119, 152-163 (1999).
14. Lapierre, C., Pollet, B., MacKay, J. J., Dimmel, D. R., Sederoff, R. R. Lignin structure in a mutant pine deficient in cinnamyl alcohol dehydrogenase. *Intern. Symp. Wood and Pulping Chem.*, Yokohama, Japan, June 7-10, 1999.
15. MacKay, J. J., Presnell, T., Jameel, H., Taneda, H., O'Malley, D. M. and Sederoff, R. R. Modified lignin properties and delignification during pulping with a mutant loblolly pine. *Holzforschung*, in press.
16. Li, J. and Gellerstedt, G. On the Structural Significance of Kappa Number Measurement. *Intern. Symp. Wood and Pulping Chem.*, Montreal, Canada, June 7-12, 1997.
17. Akin, D. E, Rigsby, L.L. (1992) Scanning electron microscopy and utraviolet absorption mirospectrophotometry of plant cell types of different biodegradabilities. Food Structure 11:259-271.
18. Fukazawa, K. (1992) Ultraviolet Microscopy. In Methods in Lignin Chemistry, Lin, S. E. and Dence, C. W. (Eds), Springer-Verlag, Berlin. p. 110-121.
19. Wu, R., O'Malley, D. M., Remington, D. L., MacKay, J. J., and McKeand, S. E. Average effect of a mutation in lignin biosynthesis in loblolly pine. *Theor. Appl. Genet.*, In press.